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(54) **STABLE, AQUEOUS ALFA INTERFERON SOLUTION FORMULATIONS**

STABILE, WÄSSRIGE ALPHA-INTERFERON LÖSUNGEN

FORMULATIONS DE SOLUTIONS AQUEUSES STABLES D'INTERFERON ALPHA

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EP 0 777 495 B1

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Description

BACKGROUND OF THE INVENTION

[0001] This invention relates to stable, aqueous solution formulations which are free of products derived from human blood serum and which maintain high biological activity and high chemical and high physical stability of alfa-type interferon for an extended period of time.

[0002] U. S. Patent 4,496,537 discloses biologically stable alfa interferon aqueous solution formulations containing alfa interferon, human serum albumin and alanine or glycine, water, and a buffer system to maintain the pH at 6.5-8.0. The human serum albumin ("HSA") acts as a stabilizer for alfa interferon and prevents losses of alfa interferon from solution by coating and/or adsorption of the alfa interferon onto the stainless steel and glass surfaces of compounding vessels, process equipment and storage containers. Solution formulations containing alfa interferon and HSA have maintained the chemical and biological stability of the alfa interferon when such solutions have been stored at 2-8°C for extended periods, i.e., more than 2 years.

[0003] Recently, the worldwide AIDS epidemic has resulted in health registration agencies requiring manufacturers to place warnings on products, such as alfa interferon, which contain products derived from human blood such as HSA .

[0004] There is a need to reformulate alfa-type interferon solution products to obtain a solution formulation free of human blood-derived products such as HSA while maintaining high chemical, high physical stability and high biological alfa-type interferon activity in the aqueous solution formulations for extended storage periods.

[0005] EP-A-0284249 describes lyophilised recombinant alpha-2 interferon preparations designed to be suitable for long term storage at ambient temperatures. These preparations require both a stabiliser and a bulking agent. Glycine, alanine and human serum albumin are listed as effective stabilisers, and human serum albumin and mannitol as effective bulking agents. Example 4 describes a solution free of human serum albumin which contain an alpha interferon, a chelating agent and 40mg/ml mannitol in which mannitol is present as a bulking agent.

[0006] WO-A-89/04177 relates specifically to gamma interferon formulations, and in particular, to the use of polyhydric sugar alcohols as stabilising agent. Typically, 40mg/ml mannitol is used to stabilise the gamma interferon.

[0007] JP 59/176,216 is concerned with providing antiseptic agents for human serum albumin-free interferon formulations.

[0008] JP 61/277633 is concerned inter alia with achieving human serum albumin-free stabilised aqueous interferon formulations and proposes the use of a broad class of surfactants, including sorbitan derivatives, for this purpose. Stabilisation of beta-interferon (examples 1 & 2) and gamma interferon (example 3) is described, but stabilisation of gamma interferon was poor as after seven days the interferon activity had declined to 59%.

[0009] JP 63/26160 relates to stabilisation of interferon-containing solutions but to partially purified natural interferon, which contains human-blood derived products.

SUMMARY OF THE INVENTION

[0010] The present invention provides a stable, aqueous solution formulation which maintains high biological alfa-type interferon activity and is free of human blood-derived products, which comprises:

a. 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon;

b. a buffer system to maintain a pH in the range of 4.5 to 7.1.

c. an effective amount of a chelating agent;

d. an amount of a stabilizer selected from polysorbate 20 and polysorbate 80 sufficient to stabilize the alfa-type interferon against loss of alfa-type interferon;

e. an effective amount of a tonicity agent;

f. an effective amount of an antimicrobial preservative; and

g. an amount of water for injection sufficient to prepare a solution of the above-listed ingredients. said formulation being free of an amount of mennitol effective to act as a bulking agent when the stabilizer is polysorbate 20.

[0011] The present invention provides a stable, aqueous solution formulation having high alfa-type interferon biological activity and free of human blood-derived products, which comprises:

a. 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon.
b. a buffer system sufficient to maintain the pH of the solution in the range of 4.5 to 7.1;
c. about 0.01 to 1 mg/mL of disodium dihydrogen ethylenediaminetetraacetate.
d. about 0.01 to 1 mg/mL of polysorbate 20 or polysorbate 80;
e. about 1 to 9 mg/mL of sodium chloride;
f. an effective amount of an antimicrobial preservative selected from m-cresol, phenol, methylparaben, propylparaben or mixtures thereof; and
g. water for injection q.s. ad. 1 mL.

[0012] In a preferred aspect, the present invention provides a stable, aqueous solution formulation having high biological alfa-type interferon activity and free of human blood-derived products, which comprises:

		mg/mL
a.	Alfa-2 Interferon	5 x 10^6 to 50 x10^6 IU
b.	Sodium Phosphate Dibasic Anhydrous	1.8
c.	Sodium Phosphate Monobasic Monohydrate	1.3
d.	Disodium Dihydrogen Ethylene-diaminetetraacetate	0.1
e.	Polysorbate 80	0.1
f.	Methylparaben	1.2
g.	Propylparaben	0.12
h.	Sodium Chloride; and	7.5
i.	Water for Injection	q.s. ad 1 mL

[0013] In another preferred aspect the present invention further provides a stabile aqueous solution formulation having high biological alfa-type interferon activity and free of human blood-derived products, which comprises:

		mg/mL
a.	Alfa-2 Interferon	5 x 10^6 to 50 x 10^6 IU
b.	Sodium Phosphate Dibasic Anhydrous	1.8
c.	Sodium Phosphate Monobasic Monohydrate	1.3
d.	Disodium Dihydrogen-Ethylenediaminetetraacetate	0.1
e.	Polysorbate 80	0.1
f.	m-Cresol	1.5
g.	Sodium Chloride; and	7.5
h.	Water for Injection	q.s. ad 1 mL

[0014] The present invention also provides a process of preparing a stable, aqueous solution formulation having high biological alfa-type interferon activity and free of human blood-derived products comprising admixing an effective amount of alfa-type interferon with a buffer system capable of maintaining the pH within the range of 4.5 to 7.1, a chelating agent, polysorbate 20 or polysorbate 80, a tonicity agent, an antimicrobial preservative and sufficient water to form a solution. In a preferred aspect of the process of the present invention, the solution is prepared and maintained substantially free of dissolved oxygen and a headspace of inert atmosphere above the solution is maintained at a value of less than about 4% by volume of oxygen.

[0015] The formulations and methods of the invention are further free of an amount of mannitol effective to act as a bulking agent.

DETAILED DESCRIPTION

[0016] We have selected specific amounts of a specific set of ingredients that have allowed us to develop an aqueous alpha-type interferon solution formulation which does not contain human serum albumin yet maintains high chemical, biological and physical stability for the alfa-type interferon on storage at 2° to 8°C for extended periods of at least 24 months.

[0017] The term "free of human blood-derived products" as used herein in reference to the formulations of the present invention means that no human blood-derived products such as HSA are used in the preparation of the solution formulations of the present invention.

[0018] The term "high chemical stability" as used herein in reference to the alfa-type interferon used in the formulations of the present invention means the alfa-type interferon maintains at least 85%, preferably 85% to 100% of its chemical integrity upon storage at 2° to 8°C for at least 24 months. See Tables 1 and 2. The chemical integrity is determined by measuring the protein content in an HPLC assay such as the one disclosed by T. L. Nagabhushan, et al., in an article entitled "Characterization of Genetically Engineered ALFA-2 Interferon", pages 79-88 appearing in Interferon Research Clinical Application, and Regulatory Consideration, Zoon, et al., eds., Elsevier Science Publishing Co., Inc. 1984. (See results in Tables 1 to 4).

[0019] The term "high biological stability" as used herein in reference to the alfa-type interferon used in the formulations of the present invention means the alfa-type interferon in the formulation maintains at least 75%, preferably at least 85%, more preferably 90% to 100% of its biological activity upon storage at 2° to 8°C for at least 24 months (see results in Tables 1 to 4) as measured in the standard method of inhibition of the cytopathic effect (CPE) of a virus such as the method disclosed by W. P. Protzman, et al., in J. Clinical Microbiology, (1985), 22, 596-599.

[0020] The term "high physical stability" as used herein in reference to the alfa-type interferon used in the formulations of the present invention means the formulation of the present invention remains clear, i.e., does not exhibit haze or visible particulate matter (i.e., particles greater than about 60 to 70 microns in diameter) on storage at 2° to 8°C for at least 24 months. See Tables 1, 2 and 3. The results listed in Tables 1, 2 and 3 are surprising in that most solution formulations containing protein products like alfa-type interferon tend to develop visually observable particulate matter (i.e., particles having diameters greater than 60 to 70 microns) upon extended storage even at 2° to 8°C. The test method used to determine particulate matter in the solution formulation of this invention (see Tables 1 to 4) is described in The United States Pharmacopeia/The National Formulary USP 23/NF 18, published by United States Pharmacopeial Convention, Inc., (1995), Rockville, Maryland; see Physical Test <788> on pages 1813 to 1816. The method used to determine the visual description of the solution formulations of this invention is also described in USP 23 as the "General. Requirement Test and Assays <1> Injections" at pages 1650 to 1652.

[0021] We have found that by adding a chelating agent to the formulations of the present invention, we have been able to avoid visible particulate matter. Typical suitable chelating agents include disodium dihydrogen ethylenediamine tetraacetate (EDTA or edetate disodium) or citric acid. The use of edetate disodium is preferred. While we do not wish to be bound by any theory, it is believed that edetate disodium effectively complexes with trace amounts of metal cations, such as Zn^{2+}, Fe^{2+}, Cu^{2+} or Al^{3+}, which ions may be present in excipients and packaging components, e.g., rubber stoppers or gaskets. Since edetate disodium has a higher affinity for these metal cations than the alfa-type interferons, the interaction between metal cations and alfa-type interferon which results in formation of insoluble complexes (in the form of, for example, visible particulate matter) and loss of activity are avoided. The effective amount of the chelating agent is in the range of 0.01 to 1 mg/mL based on 0.1×10^6 to 100×10^6 International Units ("IU") of alfa-type interferon/mL. Preferably, 0.1 mg of edetate disodium is used for 5×10^6 to 50×10^6 IU of alfa-2 interferon.

[0022] The buffer systems suitable for the formulations of the present invention are those which maintain the pH of the aqueous solution formulation in the range of 4.5 to 7.1, preferably 6.5-7.1 and most preferably 6.8. The use of a buffer system of sodium phosphate dibasic and sodium phosphate monobasic is preferred. Normally a 0.005 to 0.1 molar buffer of the preferred sodium phosphate monobasic/dibasic buffer system is used for a formulation containing 0.1×10^6 to 100×10^6 IU of alfa-type interferon per mL. Other suitable buffer systems to maintain the desired pH range of 4.5 to 7.1 include sodium citrate/citric acid and sodium acetate/acetic acid.

[0023] The tonicity agent useful in the present invention is any agent capable of rendering the formulations of the present invention iso-osmotic with human serum. Typical suitable tonicity agents include sodium chloride, mannitol, glycine, glucose and sorbitol. Use of sodium chloride as a tonicity agent is preferred.

[0024] The amount of the tonicity agent used is in the range of 1 to 10 mg/mL when the formulation of the present invention contains 0.1×10^6 to 100×10^6 IU of alfa-type interferon/mL. The use of 7.5 mg/mL of sodium chloride is preferred for 5×10^6 to 50×10^6 IU of alfa-type interferon per mL in the formulations of the present invention.

[0025] The sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivatives such as polysorbate 80 or polysorbate 20 are useful as a stabilizing agent to prevent adsorption of the alfa-type interferon proteins such as alfa-2b interferon onto the stainless steel and glass surfaces of the equipment used to make the indictable formulations containing alfa-type interferon. The amount of polysorbate 20 or 80 effective in the formulation of this invention is in the range of 0.01 to 1.0 mg per mL for a formulation containing 0.1×10^6 to 100×10^6 IU of alfa-type interferon per mL. The use of polysorbate 80 is preferred. The use of 0.1 mg/mL of polysorbate 80 is more preferred in all the solution formulations of the present invention. When the concentrations of alfa-type interferon such as alfa-2 interferon is less than about 15×10^6 IU/mL, e.g., 6×10^6 IU/mL, loss of activity due to adsorption of the alfa interferon in the absence of polysorbate 80 significantly lowers the biological activity of the formulation. Surprisingly, we have found that polysorbate 80 prevents loss of alfa-2b interferon and allows systemic delivery of the alfa-2b interferon without loss of

biological activity. In the course of development of the formulation of the present invention, we surprisingly found that polysorbate 80 provided superior chemical and biological stability to alfa-2b interferon compared to other non-ionic surfactants, e.g., Pluronic F127 and Pluronic F-68.

[0026] The amount of alfa-type interferon useful in the formulation of the present invention is in the range of 0.1 x 10^6 to 100 x 10^6 IU/mL, preferably 5 x 10^6 to 50 x 10^6 IU/mL.

[0027] The term "alfa-type interferon" as used herein means the family of highly homologous species-specific proteins that inhibit viral replication and cellular proliferation and modulate immune response. Typical suitable alfa-type interferons include interferon alfa-2a such as ROFERON A interferon alfa-2a available from Hoffmann-La Roche, Nutley, N.J., interferon alfa-2b such as INTRON A interferon alfa-2b available from Schering Corporation, Kenilworth, N.J. interferon alfa-2c such as BEROFOR interferon alfa-2c available from Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, CT., interferon alfa-n1, a purified blend of natural alfa interferons such as SUMIFERON available from Sumitomo, Japan or as WELLFERON interferon alfa-n1 available from The Wellcome Foundation Ltd., London, Great Britain, or consensus alfa interferon available from Amgen, Inc., Newbury Park, California, or interferon alfa-n3, a mixture of natural alfa interferons, made by Interferon Sciences and available from the Purdue Frederick Co., Norwalk, CT., under the ALFERON tradename. The use of interferon alfa-2a or alfa-2b is preferred. The use of interferon alfa-2b is more preferred.

[0028] The antimicrobial preservatives found useful in the present invention include m-cresol, phenol and methylparaben and propylparaben and mixtures of the above-listed preservatives, e.g., phenol-methylparaben mixtures. The effective amount of m-cresol found useful in the present invention is in the range of 0.5 to 2 mg/mL for a formulation containing 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon. It is preferred to use 1.5 mg/mL of m-cresol for a formulation containing 5 x 10^6 to 50 x 10^6 IU/mL of interferon alfa-2b.

[0029] The effective amount of phenol found useful is in the range of 0.5 to 5 mg/mL for a solution formulation containing 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon.

[0030] The effective amount of methylparaben is in the range of 0.6 to 1.8 mg/mL and the amount of propylparaben is in the range of 0.06 to 0.18 mg/mL when the formulation of the present invention contains 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon.

[0031] It is preferred to use 1.2 mg/mL of methylparaben in combination with 0.12 mg/mL of propylparaben when the formulation of the present invention contains 0.1 x 106 to 100 x 10^6 IU/mL of alfa-2b interferon.

[0032] The use of m-cresol as an antimicrobial preservative is more preferred. The water used for preparation of the formulations of the present invention is preferably water for injection.

[0033] During the course of development of the aqueous solution formulations of the present invention that would maintain high biological activity as well as high chemical and high physical stability of the alfa-type interferon over an extended storage period without employing HSA as a stabilizer, we identified that the amount of a sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl) derivative such as polysorbate 80 required to act as a stabilizing agent for the alfa-type interferon had a direct effect on the effective amount of the antimicrobial preservative which could be added to the aqueous solution formulation to provide the appropriate antimicrobial protection for said formulation pursuant to various worldwide health registration requirements without causing undesirable haze formation in the solution.

[0034] Thus, when the preferred stabilizing agent, polysorbate 80, was present in formulations of the present invention, in the preferred effective amount of 0.1 mg/mL, the effective amount of the preferred antimicrobial preservative, e.g., m-cresol which could be added without causing hazing of said formulation was found to be critical. For example if the amount of m-cresol added to a formulation which contained 0.1 mg/mL of polysorbate 80, such as shown in Example 3, is increased to greater than 1.75 mg/mL, hazing was observed. A similar hazing problem was observed when the amount of polysorbate 80 in the resultant formulation was varied from 0.01 to 1 mg/mL. No hazing was observed when 1.75 mg/mL or less, preferably about 1.5 mg/mL of m-cresol was added to a formulation prepared in accordance with the procedures of Example 3 which contains 0.1 mg/mL of polysorbate 80. This criticality was also observed with the parabens and phenol when they were used as antimicrobial preservatives. For formulations of the present invention containing 0.01 to 1 mg/mL of polysorbate 80, the effective amount of methylparaben should be no more than about 1.2 mg/mL when used with 0.12 mg/mL of propylparaben to avoid hazing, and the effective amount of phenol (when it is used in place of the parabens) should be in the range of 0.5 to less than about 4 mg/mL to avoid hazing.

[0035] Alfa-type interferon formulations are useful for treatment of a variety of disease states such as renal cell carcinomas, AIDS-related Kaposi's sarcoma, chronic and acute hepatitis B, chronic and acute non-A, non-B/C hepatitis. The formulations of the present invention are useful in treating these disease states preferably as injectable aqueous solutions.

EXAMPLES

[0036] The following non-limiting examples illustrate the preparation of the aqueous solutions of alfa-type interferons.

[0037] The procedures listed after Example 5 are used to prepare the formulations of the present invention of Examples 1 to 5.

EXAMPLE 1		
ActiveSubstance:	Interferon alfa-2b	0.1 x 10^6-100 x 10^6 IU/mL*
Buffer:	Sodium Phosphate (monobasic/dibasic)	0.005-0.1 M
Chelating Agent:	Edetate Disodium	0.01 - 1 mg/mL
Stabilizer: Tonicity Adjusting	Polysorbate 80	0.01 - 1 mg/mL
Agent: Antimicrobial	Sodium Chloride	1 - 9 mg/mL
Preservative:	m-Cresol	0.5 -1.75 mg/mL
	or Phenol or Methylparaben	0.5 - <4 mg/mL
	Propylparaben	0.6 - 1.2 mg/mL
		0.06 - 0.12 mg/mL
Solvent:	Water for Injection q.s. ad	1 mL

*IU-International Units

EXAMPLE 2	
Interferon alfa-2b	10 X 10^6 IU/mL
Sodium Phosphate Dibasic Anhydrous	1.8 mg/mL
Sodium Phosphate Monobasic Monohydrate	1.3 mg/mL
Edetate Disodium	0.1 mg/mL
Polysorbate 80	0.1 mg/mL
Methylparaben	1.2 mg/mL
Propylparaben	0.12 mg/mL
Sodium Chloride	7.5 mg/mL
Water for Injection q.s. ad	1 mL

Example 3	
Interferon alfa-2b	10 x 10^6 IU/mL
Sodium Phosphate Dibasic Anhydrous	1.8 mg/mL
Sodium Phosphate Monobasic Monohydrate	1.3 mg/mL
Edetate Disodium	0.1 mg/mL
Polysorbate 80	0.1 mg/mL
m-Cresol	1.5 mg/mL
Sodium Chloride	7.5 mg/mL
Water for Injection q.s. ad	1 mL

[0038] Stability data on Examples 2 and 3 are summarized in Tables 1 and 2 respectively.

EXAMPLE 4

[0039] The formulation of Example 3 was prepared with 6 x 10^6 IU/mL of alfa-2b interferon in accordance with the method of manufacture detailed herein below using nitrogen sparging of the solution and maintaining no more than about 4% by volume of oxygen in the headspace.

[0040] Vials containing a label volume of 3 mL of solution were stored at 30°, 25° and 4°C. The results are summarized in Table 3.

EXAMPLE 5

[0041] The formulation of Example 4 was prepared in accordance with the method of manufacture detailed hereinbelow in all details except no nitrogen was sparged through the solution or overlaid upon it and the oxygen volume in

the headspace was ~20% by volume as found in ambient air.

[0042] Vials containing a label volume of 3 mL of solution w re stored at 30°, 25° and 4°C. The results are summarized in Table 4.

[0043] Similar results are expected if the interferon alfa-2b in Examples 1 to 5 is substituted by an equivalent amount of Roferon A, Wellferon or Sumiferon interferon alfa.

Table 1

Interferon Alfa-2b Stability Data on Example 2

Time (month)	Temp (°C)	Anti-Viral Assay (CPE) ($x10^6$ IU/mL)	(%L.S.)	Protein Content (HPLC Assay) (mcg/mL)	(% of initial)	Particulate Matter (particles/container) ≥10 µ	≥25 µ	≥50 µ	Description
Initial		10.0	100	42.5	100	40	3	1	ccs*
3	4	9.0	90	42.4	100	16	13	11	ccs
6	4	10.0	100	41.8	98	8	3	1	ccs
9	4	10.0	100	43.3	102	52	3	0	ccs
12	4	10.0	100	44.3	104	17	4	2	ccs
18	4	9.8	98	41.5	98	6	1	0	ccs
24	4	10.0	100	39.5	93	5	1	0	ccs

* ccs - clear, colorless solution, essentially free of visible particles.

EP 0 777 495 B1

Table 2

Interferon Alfa 2b Stability Data on Example 3

Time (month)	Temp (°C)	Anti-Viral Assay ($x10^6$ IU/mL)	(%L.S.)	Protein Content (HPLC Assay) (mcg/mL)	(% of Initial)	Particulate Matter (particles/container) ≥ 10 µ	≥ 25 µ	≥ 50 µ	Description
Initial		10.3	103	37.9	100	68	4	3	ccs*
1	4	10	100	38.2	101	142	24	23	ccs
3	4	10	100	38.9	103	311	63	35	ccs
6	4	10	100	40.0	105	206	17	16	ccs
9	4	10	100	37.1	97.9	211	109	50	ccs
12	4	10	100	36.6	96.6	300	65	12	ccs
15	4	10	100	36.1	95.3	123	8	6	ccs

* ccs - clear, colorless solution, essentially free of visible particles.

Table 3

Interferon Alfa 2b Stability Data on Example 4

Time (month)	Temp (°C)	Position of Vial*	Anti-Viral Assay (CPE) (x10^{6}IU/mL)	Anti-Viral Assay (CPE) (%L.S.)	Protein Content (HPLC Assay) (mcg/mL)	Protein Content (HPLC Assay) (% of Initial)	m-Cresol Assay (mg/mL)	m-Cresol Assay %LS	pH
	Initial		6.48	108	25.7	100	1.47	98.0	6.91
1	30	UP	6.00	100	24.8	96.5	1.47	98.0	6.90
		INV	6.00	100	24.8	96.5	1.47	98.0	6.90
3	4	UP	6.00	100	23.5	91.4	1.46	97.3	6.88
		INV	6.00	100	24.2	94.2	1.48	98.7	6.87
3	25	UP	6.00	100	21.7	84.4	1.43	95.3	6.88
		INV	6.00	100	21.7	84.4	1.47	98.0	6.88
6	4	UP	6.00	100	24.6	95.7	1.46	97.3	6.84
		INV	6.00	100	24.3	94.6	1.45	96.7	6.84
	25	UP	5.56	92.3	20.5	79.8	1.45	96.7	6.85
		INV	6.00	100	20.4	79.4	1.44	96.0	6.85
12	4	UP	6.00	100	23.4	91.0	1.47	98.0	6.84
		INV	6.00	100	23.4	91.0	1.46	97.3	6.84

* UP - Upright
INV - Inverted

Table 3 - Continued.

Time (month)	Temp (°C)	Position of Vial*	Particulate Matter No. of particles/vial ≥10µm	≥25µm	≥50µm	Description
Initial			23	2	0	CCS*
1	30	UP	109	61	16	CCS
		INV	55	11	2	CCS
3	4	UP	29	2	0	CCS
		INV	59	23	2	CCS
3	25	UP	141	76	17	CCS
		INV	49	16	3	CCS
6	4	UP	59	21	3	CCS
		INV	68	20	4	CCS
	25	UP	38	4	0	CCS
		INV	57	8	0	CCS
12	4	UP	21	2	0	CCS
		INV	18	2	0	CCS

* CCS - Clear, colorless solution, essentially free of visible particles.

Table 4

Interferon Alfa-2b Stability Data for Example 5

Time (month)	Temp (°C)	Position of Vial	Anti-Viral Assay (CPE)		Protein Content (HPLC Assay)		m-Cresol Assay		
			($x10^6$ IU/mL)	(% L.S.)	(µg/mL)	(% of Initial)	(mg/mL)	% L.S	pH
Initial			6.00	100	25.5	100	1.47	98.0	6.85
1	30	UP	6.00	100	19.5	76.5	1.49	99.3	6.82
		INV	6.00	100	19.5	76.5	1.50	100	6.83
3	4	UP	6.00	100	24.0	94.1	1.43	95.3	6.81
		INV	6.00	100	24.0	94.1	1.43	95.3	6.82
3	25	UP	6.00	100	20.2	79.2	1.39	92.7	6.82
		INV	6.00	100	19.6	76.9	1.41	94.0	6.82
6	4	UP	6.00	100	24.6	96.5	1.47	98.0	6.82
		INV	6.00	100	24.6	96.5	1.47	98.0	6.83
	25	UP	6.00	100	17.2	67.5	1.47	98.0	6.83
		INV	6.00	100	16.1	63.1	1.48	98.7	6.84
12	4	UP	7.56	126	23.3	91.4	1.58	105	6.88
		INV	7.00	117	23.2	91.0	1.41	94.0	6.88

EP 0 777 495 B1

Table 4 - Continued

Time (month)	Temp (°C)	Position of Vial	Particulate Matter No. of particles/vial ≥ 10µm	≥ 25µm	≥ 50µm	Description
	Initial		144	4	4	CCS*
1	30	UP	89	3	1	CCS
		INV	64	1	0	CCS
3	4	UP	39	1	0	CCS
		INV	77	19	6	CCS
3	25	UP	57	1	0	CCS
		INV	140	24	1	CCS
6	4	UP	66	1	0	CCS
		INV	220	87	27	CCS
	25	UP	92	3	0	CCS
		INV	241	5	0	CCS
12	4	UP	63	1	0	CCS
		INV	119	6	1	CCS

*CCS - Clear, colorless solution, essentially free of visible particles.

Method of Manufacture for Examples 1 to 5

A. Compounding Paraben-Containing Aqueous Solution

Formulations Such as Shown in Example 2

[0044]

1. Charge approximately 80% of the water for injection at a temperature greater than 70°C into a suitable jacketed compounding vessel equipped with an agitator.

2. Separately charge approximately 30% of the water for injection into another suitable vessel. Cool and maintain the water temperature between 20° and 25°C. Begin sparging and overlaying the water which will be used to bring the batch to final volume with filtered nitrogen to maintain a dissolved oxygen level at or below 0.25 ppm.

3. Charge and dissolve with mixing methylparaben and propylparaben into the compounding vessel in step 1 while maintaining the temperature of the solution between 70° and 80°C.

4. Cool the solution in step 3 to a temperature between 20° and 25°C. Sparge and overlay the solution with filtered nitrogen. Maintain a dissolved oxygen level at or below 0.25 ppm.

5. Charge and dissolve with mixing the following ingredients into the solution in step 4 while maintaining nitrogen sparging and overlaying:

Sodium phosphate dibasic anhydrous
Sodium phosphate monobasic monohydrate
Edetate disodium
Sodium chloride

6. Discontinue nitrogen sparging of the solution from step 5. Maintain nitrogen overlaying in the compounding vessel.

7. Charge and dissolve polysorbate 80 in approximately 50 mL of water for injection (for a 1-liter size batch) in a separate vessel. Transfer the polysorbate 80 solution into the solution from step 6.

8. Check the pH of the solution. It should be between 6.6 and 7.0. No pH adjustment is required.

9. Charge interferon alfa-2b bulk drug solution into the solution in step 8 while mixing.

10. Add water for injection that has been sparged with nitrogen (from step 2) to bring batch to final volume. Agitate solution gently until homogeneous.

11. Aseptically filter the solution through a sterilized filter that has been washed and tested for integrity. Collect the sterilized solution into a sterilized filling vessel that has been overlaid with sterile-filtered nitrogen. Integrity test the filter after filtration.

12. Overlay filling vessel in step 11 with sterile-filtered nitrogen and seal.

B. Compounding m-Cresol-Containing Aqueous Solution

Formulations Such as Shown in Example 3

[0045] The manufacturing procedure used to prepare the aqueous solutions containing m-cresol as a preservative (such as shown in Example 3) is exactly the same as described hereinabove except the temperature of the solution in Step 3 is maintained between 20° and 25°C and the m-cresol is charged after Step 6.

C. Compounding HSA-Free Aqueous Alfa Interferon Solution Formulations Under Ambient Air

[0046] The manufacturing procedure used to prepare the HSA-free aqueous alfa interferon formulations of Examples 1 to 4 was used to prepare the formulations such as that of Example 5 except that all the steps were performed under ambient air; no nitrogen was sparged through the solution or overlaid upon it and ambient air (normally containing about 20% by volume of oxygen) occupied the headspace volume.

[0047] To maintain high chemical, physical and biological stability, it is preferred that the water used to prepare the aqueous alfa interferon solution as well as the so-formed aqueous alfa interferon solution be substantially free of dissolved oxygen, and the aqueous solution be made and stored with a headspace of an inert atmosphere, such as nitrogen, containing no more than about 4% volume of oxygen. By the term "substantially free of dissolved oxygen" as used herein is meant an oxygen level of no more than about 0.25 ppm at a water temperature of about 20° - 25°C. Normally, this preferred dissolved oxygen level of 0.25 ppm is conveniently achieved by sparging an inert atmosphere, e.g. nitrogen gas into the water used to prepare the aqueous solutions (maintained at a temperature of about 20° - 25°C) for a time sufficient (e.g. about 30 minutes) to lower the dissolved oxygen to a value of no more than about 0.25 ppm. The sparging is continued throughout the manufacturing procedure to maintain the dissolved oxygen level at 0.25 ppm. We have found that aqueous formulations of the present invention which have a dissolved oxygen level of 1ppm and an oxygen content in the headspace of 7% by volume demonstrated significantly greater loss of chemical stability of the alfa interferon after 3 months of storage at 25°C compared to a similar aqueous formulation having the preferred dissolved oxygen level of 0.25 ppm and an oxygen content in the headspace of 4% by volume stored under the same conditions.

[0048] A side-by-sid comparison of the alfa-2b interferon solution stability data shown in Tables 3 and 4 shows that there is no significant stability difference between the aqueous solution formulations of the present invention which were prepared under the nitrogen/low oxygen conditions used in Example 4 and those prepared in accordance with Example 5 under ambient air during storage for 12 months at 4°C. In contrast, a comparison of the alfa-2b interferon stability in solutions of Examples 4 and 5 stored at higher temperatures, e.g, 25° and 30°C shows the protective effect achieved by the preferred (safer) use of nitrogen sparging to effect low dissolved oxygen levels in the aqueous solution while simultaneously maintaining an oxygen content in the headspace at a value of no more than about 4% by volume.

[0049] The aqueous solution formulation of the present invention may be stored in any suitable washed and sterilized filing vessels or container such as 2-mL or 5-mL Type I flint glass vials stoppered with gray butyl rubber closures. The aqueous solution formulations of the present invention may also be stored in prefilled multi-dose syringes such as those useful for delivery of indictable solutions of drugs such as insulin. Typical suitable syringes include systems comprising a prefilled vial attached to a pen-type syringe such as the Novolet Novo Pen available from Novo Nordisk. Typical suitable systems include a prefilled, pen-type syringe which allows easy self-injection by the user as well as accurate, reproducible dose settings.

[0050] The aqueous solutions formulations of the present invention, such as present in the Examples may also be lyophilized to form a powder for reconstitution. The lyophilized alfa-type interferon powder is expected to maintain its chemical and biological stability when stored at 2° to 8°C for at least 2 years.

Claims

1. A stable, aqueous formulation having high biological alfa-type interferon activity and free of human blood-derived products which comprises:

   a. 0.1 x 10^6 to 100 x 10^6 IU/mL of alfa-type interferon;

   b. a buffer system to maintain a pH in the range of 4.5 to 7.1;

   c. an effective amount of a chelating agent;

   d. an amount of a stabilizer selected from polysorbate 20 and polysorbate 80 sufficient to stabilize the alfa-type interferon against loss of alfa-type interferon;

   e. an effective amount of a tonicity agent;

   f. an effective amount of an antimicrobial preservative; and

   g. an amount of water for injection sufficient to prepare a solution of the above-listed ingredients;

   said formulation being free of an amount of mannitol effective to act as a bulking agent when the stabilizer is polysorbate 20.

2. The composition of claim 1 wherein the buffer system is sodium dibasic phosphate and sodium monobasic phosphate.

3. The composition of claim 1 wherein the chelating agent is edetate disodium or citric acid.

4. The composition of claim 1 wherein the tonicity agent is sodium chloride.

5. The composition of claim 1 wherein said preservative is selected from m-cresol, phenol, methylparaben, propylparaben or mixtures thereof.

6. The composition of claim 1 wherein the alfa-type interferon is interferon alfa-2.

7. A stable aqueous solution formulation having high biological interferon alfa-2 activity and free of human serum albumin, which comprises:

		mg/mL
a.	Interferon alfa-2	5 X 10^6 to 50 x 10^6 IU
b.	Sodium Phosphate Dibasic Anhydrous	1.8
c.	Sodium Phosphate Monobasic Monohydrate	1.3
d.	Disodium Dihydrogen Ethylenediamine tetraacetate	0.1
e.	A stabilizer selected from polysorbate 20 and polysorbate 80	0.1
f.	Methylparaben	1.2
g.	Propylparaben	0.12
h.	Sodium Chloride and	7.5
i.	Water for Injection	q.s. ad 1 mL

8. A stable, aqueous solution formulation having high biological interferon alfa-2 activity and free of human serum albumin, which comprises:

		mg/mL
a.	Interferon alfa-2	5 X 10^6 to 50 x 10^6 IU
b.	Sodium Phosphate Dibasic Anhydrous	1.8
c.	Sodium Phosphate Monobasic Monohydrate	1.3
d.	Disodium Dihydrogen Ethylenediamine tetraacetate	0.1
e.	A stabilizer selected from polysorbate 20 and polysorbate 80	0.1
f.	m-Cresol	1.5
g.	Sodium Chloride and	7.5
h.	Water for Injection	q.s. ad 1 mL

9. An article of manufacture comprising a packaging material and the formulation of any preceding claim wherein said packaging material is a multidose glass vial.

10. An article of manufacture comprising a prefilled syringe containing an effective amount of the formulation of any of Claims 1 to 8.

11. An article of manufacture comprising a packaging material and the formulation of any of Claims 1 to 8 wherein said packaging material is a single-dose vial.

12. A process of preparing a stable, aqueous formulation having high biological alfa-type interferon activity and free of human blood derived products comprising admixing an effective amount of alfa-type interferon with a buffer system capable of maintaining the pH within the range of 4.5 to 7.1, a chelating agent, a stabilizer selected from polysorbate 20 and polysorbate 80, a tonicity agent, an antimicrobial preservative and sufficient water to make an aqueous solution, wherein when the stabilizer is polysorbate 20 the method is carried out in the absence of an amount of mannitol effective to act as a bulking agent.

13. The process of claim 12 wherein the aqueous solution is prepared and maintained substantially free of dissolved oxygen.

14. The process of Claim 13, wherein the dissolved oxygen is no more than 0.25 ppm.

15. The process of Claim 13 or 14, wherein the headspace above the aqueous solution is an inert atmosphere containing no more than 4% by volume oxygen.

16. A sterilized filling vessel containing a formulation obtainable by a process as claimed in any of Claims 12 to 15.

Patentansprüch

1. Stabile wäßrige Zubereitung, die eine hohe biologische Aktivität von Interferon des α-Typs aufweist und frei von Produkten ist, die von menschlichem Blut abgeleitet sind, und die folgendes umfaßt:

   a. 0,1 x 10^6 bis 100 x 10^6 IE/ml Interferon des α-Typs;

   b. ein Puffersystem, das den pH-Wert im Bereich von 4,5 bis 7,1 hält;

   c. eine effektive Menge eines Chelatisierungsmittels;

   d. eine zur Stabilisierung des Interferons des α-Typs gegenüber Verlust an Interferon des α-Typs ausreichende Menge eines Stabilisators, der aus Polysorbat 20 und Polysorbat 80 ausgewählt ist;

   e. eine effektive Menge eines Tonizitätsmittels;

   f. eine effektive Menge eines antimikrobiellen Konservierungsmittels; und

   g. eine zur Herstellung einer Lösung der oben aufgeführten Bestandteile ausreichende Menge Wasser für die Injektion;

   wobei die Zubereitung frei von einer Menge Mannit ist, die als Füllstoff wirken kann, wenn der Stabilisator Polysorbat 20 ist.

2. Zusammensetzung gemäß Anspruch 1, wobei es sich bei dem Puffersystem um Natriumdihydrogenphosphat und Natriummonohydrogenphosphat handelt.

3. Zusammensetzung gemäß Anspruch 1, wobei es sich bei dem Chelatisierungsmittel um Dinatrium-EDTA oder Zitronensäure handelt.

4. Zusammensetzung gemäß Anspruch 1, wobei es sich bei dem Tonizitätsmittel um Natriumchlorid handelt.

5. Zusammensetzung gemäß Anspruch 1, wobei das Konservierungsmittel aus m-Kresol, Phenol, Methylparaben, Propylparaben oder Gemischen davon ausgewählt ist.

6. Zusammensetzung gemäß Anspruch 1, wobei es sich bei dem Interferon des α-Typs um Interferon α-2 handelt.

7. Stabile Zubereitung in Form einer wäßrigen Lösung, die eine hohe biologische Aktivität von Interferon α-2 aufweist und frei von Humanserumalbumin ist und die folgendes umfaßt:

		mg/ml
a.	Interferon α-2	5 x 10^6 bis 50 x 10^6 IE
b.	wasserfreies Natriumdihydrogenphosphat	1,8
c.	Natriummonohydrogenphosphat-Monohydrat	1,3
d.	Dinatriumdihydrogenethylendiamintetraacetat	0,1
e.	einen Stabilisator, der aus Polysorbat 20 und Polysorbat 80 ausgewählt ist;	0,1
f.	Methylparaben	1,2
g.	Propylparaben	0,12
h. und	Natriumchlorid	7,5
i.	Wasser für die Injektion	q.s. ad 1 ml.

8. Stabile Zubereitung in Form einer wäßrigen Lösung, die eine hohe biologische Aktivität von Interferon α-2 aufweist und frei von Humanserumalbumin ist und die folgendes umfaßt:

		mg/ml
a.	Interferon α-2	5×10^6 bis 50×10^6 IE
b.	wasserfreies Natriumdihydrogenphosphat	1,8
c.	Natriummonohydrogenphosphat-Monohydrat	1,3
d.	Dinatriumdihydrogenethylendiamintetraacetat	0,1
e.	einen Stabilisator, der aus Polysorbat 20 und Polysorbat 80 ausgewählt ist;	0,1
f.	m-Kresol	1,5
g. und	Natriumchlorid	7,5
h.	Wasser für die Injektion	q.s. ad 1 ml.

9. Industriell gefertigtes Produkt, das ein Verpackungsmaterial sowie die Zubereitung gemäß einem der vorstehenden Ansprüche umfaßt, wobei das Verpackungsmaterial eine Mehrfachdosis-Glasampulle ist.

10. Industriell gefertigtes Produkt, das eine vorgefüllte Spritze umfaßt, die eine effektive Menge der Zubereitung gemäß einem der Ansprüche 1 bis 8 enthält.

11. Industriell gefertigtes Produkt, das ein Verpackungsmaterial sowie die Zubereitung gemäß einem der Ansprüche 1 bis 8 umfaßt, wobei das Verpackungsmaterial eine Einzeldosis-Ampulle ist.

12. Verfahren zur Herstellung einer stabilen wäßrigen Zubereitung, die eine hohe biologische Aktivität von Interferon des α-Typs aufweist und frei von Produkten ist, die von menschlichem Blut abgeleitet sind, umfassend das Mischen einer effektiven Menge Interferon des α-Typs mit einem Puffersystem, das den pH-Wert im Bereich von 4,5 bis 7,1 zu halten vermag, einem Chelatisierungsmittel, einem Stabilisator, der aus Polysorbat 20 und Polysorbat 80 ausgewählt ist, einem Tonizitätsmittel, einem antimikrobiellen Konservierungsmittel und genügend Wasser, um eine wäßrige Lösung herzustellen, wobei das Verfahren in Abwesenheit einer Menge Mannit, die als Füllstoff wirken kann, durchgeführt wird, wenn der Stabilisator Polysorbat 20 ist.

13. Verfahren gemäß Anspruch 12, wobei die wäßrige Lösung im wesentlichen frei von gelöstem Sauerstoff hergestellt und gehalten wird.

14. Verfahren gemäß Anspruch 13, wobei die Menge des gelösten Sauerstoffs nicht größer ist als 0,25 ppm.

15. Verfahren gemäß Anspruch 13 oder 14, wobei der Kopfraum über der wäßrigen Lösung eine inerte Atmosphäre ist, die nicht mehr als 4 Vol.-% Sauerstoff enthält.

16. Sterilisiertes Füllgefäß, das eine Zubereitung enthält, die durch ein Verfahren gemäß einem der Ansprüche 12 bis 15 erhalten werden kann.

Revendications

1. Formulation aqueuse stable ayant une activité d'interféron du type alpha biologique élevée et sans produits dérivés du sang humain, qui comprend :

a. $0,1 \times 10^6$ à 100×10^6 UI/ml d'interféron du type alpha ;
b. un système tampon pour maintenir un pH de l'ordre de 4,5 à 7,1 ;
c. une quantité efficace d'un agent de chélation ;
d. une quantité d'un stabilisant sélectionné parmi le polysorbate 20 et le polysorbate 80, suffisante pour stabiliser l'interféron du type alpha contre une perte de l'interféron du type alpha ;
e. une quantité efficace d'un agent de tonicité ;
f. une quantité efficace d'un conservateur antimicrobien ; et
g. une quantité d'eau pour injection suffisante pour préparer une solution des ingrédients dont la liste est donnée ci-dessus ;

ladite formulation étant exempte d'un quantité de mannitol efficace pour agir en tant qu'agent gonflant quand le stabilisant est polysorbate 20.

2. Composition de la revendication 1, où le système tampon est du phosphate de sodium dibasique et du phosphate de sodium monobasique.

3. Composition de la revendication 1, où l'agent de chélation est l'édétate disodique ou l'acide citrique.

4. Composition de la revendication 1 où l'agent de tonicité est du chlorure de sodium.

5. Composition de la revendication 1, où ledit conservateur est sélectionné parmi m-crésol, phénol, méthylparabène, propylparabène ou leurs mélanges.

6. Composition de la revendication 1, où l'interféron du type alpha est l'interféron alpha-2.

7. Formulation de solution aqueuse stable ayant une activité d'interféron alpha-2 biologique élevée et sans albumine de sérum humain, qui comprend :

		mg/ml
a.	Interféron alpha-2	5×10^6 à 50×10^6 UI
b.	Phosphate de sodium dibasique anhydre	1,8
c.	Phosphate de sodium monobasique monohydraté	1,3
d.	Dihydrogéno éthylènediamine tétraacétate disodique	0,1
e.	Un stabilisant sélectionné parmi polysorbate 20 et polysorbate 80	0,1
f.	Méthylparabène	1,2
g.	Propylparabène	0,12
h.	Chlorure de sodium et	7,5
i.	Eau pour injection	sq à 1 ml

8. Formulation de solution aqueuse stable ayant une activité d'interféron alpha-2 biologique élevée et sans albumine de sérum humain, qui comprend :

	mg/ml	
a.	Interféron alpha-2	5×10^6 à 50×10^6 UI
c.	Phosphate de sodium dibasique anhydre	1,8
c.	Phosphate de sodium monobasique monohydraté	1,3
d.	Dihydrogéno éthylènediamine tétraacétate disodique	0,1
e.	Un stabilisant sélectionné parmi polysorbate 20 et polysorbate 80	0,1
f.	m-crésol	1,5
g.	Chlorure de sodium et	7,5
h.	Eau pour injection	sq à 1 ml

9. Article de manufacture comprenant un matériau d'emballage et la formulation de toute revendication précédente, où ledit matériau d'emballage est une fiole en verre multidose.

10. Article de manufacture comprenant une seringue préremplie contenant une quantité efficace de la formulation de l'une des revendications 1 à 8.

11. Article de manufacture comprenant un matériau d'emballage et la formulation de l'une des revendications 1 à 8, où ledit matériau d'emballage est une fiole à une seule dose.

12. Procédé de préparation d'une formulation aqueuse stable ayant une activité d'interféron du type alpha biologique

élevée, et sans produits dérivés du sang humain, comprenant le mélange d'une quantité efficace d'interféron du typ alpha avec un système tampon capable de maintenir le pH dans la gamme de 4,5 à 7,1, un agent de chélation, un stabilisant sélectionné parmi polysorbate 20 et polysorbate 80, un agent de tonicité, un conservateur antimicrobien et suffisamment d'eau pour former une solution aqueuse, où quand le stabilisant est polysorbate 20, la méthode est effectuée en l'absence d'une quantité de mannitol efficace pour servir d'agent gonflant.

13. Procédé de la revendication 12, où la solution aqueuse est préparée et maintenue sensiblement sans oxygène dissous.

14. Procédé de la revendication 13, où l'oxygène dissous ne représente pas plus de 0,25 ppm.

15. Procédé de la revendication 13 ou 14, où l'espace de tête au-dessus de la solution aqueuse est une atmosphère inerte ne contenant pas plus de 4% en volume d'oxygène.

16. Récipient de remplissage stérilisé contenant une formulation pouvant être obtenue par un procédé selon l'une quelconque des revendications 12 à 15.